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PATENT
Docket No. 212302000320

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

Stefan MILTENYI et al.

Serial No.: 08/416,920

Filing Date: April 21, 1995

For: DIRECT SELECTION OF CELLS BY SECRETION PRODUCT

Examiner: R. Schwadron

Group Art Unit: 1644

DECLARATION OF MARIO ASSENMACHER PURSUANT TO 37 C.F.R § 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

I, Mario Assenmacher, declare as follows:

1. I am currently Research & Development Manager at Miltenyi Biotec GmbH., Assignee of the above-identified patent application. My Curriculum Vitae is attached as Exhibit 1.

2. I am an expert in the field of Immunology, especially of cytokine and T cell biology. I have read the above-identified application and have reviewed the pending claims. I have also read the Advisory Action dated March 17, 2000.

3. The present specification discloses methods to positively identify cells based on a product secreted by the cells and methods to positively separate cells secreting a product. The methods are used to distinguish and separate product-producing cells from non-product producing cells. Incubation conditions for the methods to positively identify and separate cells are such that a product secreted by a product-producing cell is essentially not captured by another cell. A variety of incubation conditions influence the methods and include, for example,

the time of secretion of the product from the cell, that is, incubation time; cell concentration; the level of secretion of the product; and the affinity of the capture moiety for the product.

4. With respect to incubation medium, as taught in the specification, the methods to positively identify cells and positively separate product-producing cells can be performed with medium that does not contain a substance which slow diffusion. Optionally, the incubation medium may include a substance which slows diffusion. The use of incubation medium that contains a substance that slows diffusion depends upon the other incubation conditions that influence the methods. A determination of incubation conditions for a particular set of experimental conditions is within the skill of one in the art.

5. Example 1 describes an experiment wherein a mixture of IgM-secreting cells (B1.8) and non-IgM-secreting cells (X63) were subjected to a method of identifying using medium without a high viscosity or gel forming medium and analyzed after different duration of secretion time, that is, after 10 minutes, 30 minutes, 1 hour and 2 hours. As shown in the specification in Figure 6b, two populations were differentiated after 30 minutes. Therefore, methods to positively identify cells and to positively separate cells can be successfully performed in the absence of high viscosity or gel forming medium.

6. Incubation conditions for the methods of the present invention will vary depending upon the experimental conditions used, such as for example, the number of secreting cells within a given cell population; the level of secretion of the product; and the affinity of the capture moiety for the product. Determining incubation conditions for particular experimental conditions in order to distinguish product producing cells from non-product producing cells is well within the skill of one in the art.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

21. June 2001
Date

Mario Assenmacher